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DegP: a Protein "Death Star"

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DOI 10.1016/j.str.2008.06.004

DegP is both an ATP-independent protease and chaperone in the *E. coli* periplasm. In a new structural model of DegP published in *Nature*, Krojer et al. suggest that DegP carries out these seemingly opposing roles by assembling into enormous spherical multimers.

The *E. coli* cell envelope (which consists of the cytoplasmic and outer membranes and the soluble periplasmic compartment that is bounded by them) and the extracellular space around mammalian cells share a similar problem: there isn't any ATP to assist proteases in degrading misfolded proteins or chaperones in promoting protein folding. However, the accumulation of misfolded proteins in these compartments is thought to be toxic and in humans has been implicated in causing disease (Betton et al., 1998; Nelson and Eisenberg, 2006). In both prokaryotes and eukaryotes, DegP (HtrA in mammalian cells) can function as both a protease and a chaperone without the assistance of ATP (Clausen et al., 2002). In *E. coli*, DegP is an important periplasmic protease that degrades misfolded proteins and is upregulated by both the Cpx and σ^E protein quality control pathways under conditions of protein folding stress (Ruiz and Silhavy, 2005). In addition, DegP can operate as a chaperone to assist the folding of some proteins in the periplasm (Spiess et al., 1999). However, the molecular mechanism by which DegP alternates between these two roles is unknown.

DegP is normally hexameric in solution, and the previously published X-ray crystal structure of DegP shows that the hexamer ($DegP_6$) is composed of a dimer of two trimeric rings (Krojer et al., 2002). The proteolytic active sites are oriented toward an aqueous channel between the two trimers, and the two PDZ domains of each monomer point out into the solution. However, this structure presented two mysteries: The first is that the aqueous channel at the interface of the two trimers is large enough to accommodate completely unfolded substrates, but it was difficult to imagine how DegP could accommodate partially folded or even aggregated substrates in its capacity as either a chaperone or a protease. The second mystery is that the catalytic triad in this structure is completely distorted, which should render the protease inactive.

The most recent work presented by Krojer et al., (2008) makes it clear that the $DegP_6$ is probably the resting form of the protein. The active forms of the protein are actually massive 12-mer and 24-mer spherical multimers ($DegP_{12}$ and $DegP_{24}$, respectively), which the authors solved in a 3 Å resolution crystal structure and by cryo-electron microscopy. The trick in obtaining the active conformation was to incubate catalytically inactive DegP with its physiological substrates, which the authors identified as predominantly outer membrane proteins (OMPs). Interaction with substrate appears to trigger DegP trimers to assemble into huge spherical $DegP_{12}$ and $DegP_{24}$ multimers, primarily through interactions between the PDZ domains. Moreover, interaction with substrate leads to a local repositioning of the catalytic loops into a proteolytically active conformation and causes a 15-fold increase in the protease activity toward chromogenic substrate. Thus, in the presence of misfolded substrates, DegP assembles into miniature protein "Death Stars", which completely encapsulate and degrade misfolded substrates.

However, the function of these Death Stars isn't limited to protein degradation. The $DegP_{12}$ and $DegP_{24}$ structures suggest that they assist the initial folding of OMPs by completely encapsulating and sequestering them away from the periplasm. At around 700,000 $Å^3$ (roughly the size of a 300 kDa globular protein), the central cavity of $DegP_{24}$ is nearly 8-fold larger than that of the cytoplasmic chaperonin GroEL and more than sufficient to enclose even the largest of OMPs. The authors propose that this is exactly what happens since OMPs are not only stable in the presence of catalytically active DegP but are actually protected by DegP from proteolysis by externally added proteases in vitro. While misfolded proteins could have flexible loops that make themselves available to the proteolytic active sites, the structure of OMP protomers might intrinsically protect them from the proteolytic "laser beam" of the Death Star. In support of this model, the authors observe an electron-dense area in the central cavity of $DegP_{12}$ multimers in cryo-electron microscopy, the size and shape of which suggests that it is a folded OMP protomer.

DegP may even play a role in OMP insertion into the outer membrane. The

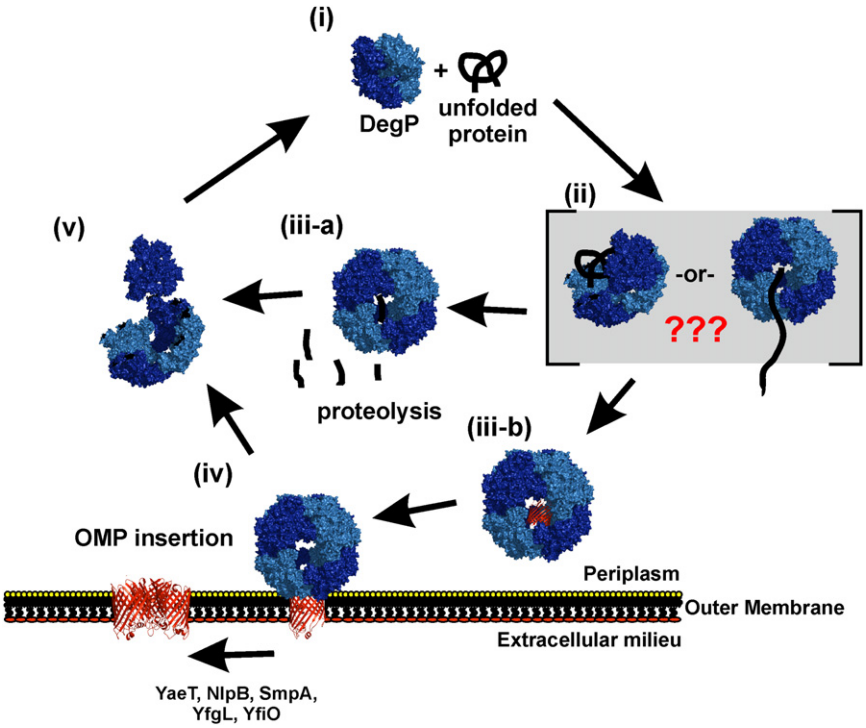

Figure 1. Schematic Model of Proposed DegP Catalytic Cycle

See text for a detailed description of the catalytic cycle. The cycle starts at step (i). DegP trimers are colored in alternating dark and light blue to enhance visualization. The DegP hexamer in step (i) is depicted in the "closed" conformation (Krojer et al., 2002). The 24-mer structure is depicted in all other steps (Krojer et al., 2008). The OmpC structures were generated from file 2J1N (Basle et al., 2006). All structural models were generated using PyMol v0.99.

largest pores leading into the DegP central cavity are lined with positively charged residues, which the authors propose are important for interaction with the negatively charged surface of the outer membrane. Indeed, mutations in these residues reduce the ability of DegP to bind membranes in vitro.

While most cytoplasmic proteases assemble into stable multimers, Krojer et al. propose that DegP differs significantly from their example by regulating its activity through a dynamic equilibrium between its multimeric states. We've schematically represented a model for the DegP catalytic cycle in Figure 1: (i) $DegP_6$ traps unfolded or misfolded substrate either through interactions with its hydrophobic internal surface or with its PDZ domains. This interaction concomitantly (ii) destabilizes the trimer-trimer interface and promotes assembly of $DegP_3$ into $DegP_{12}$ and $DegP_{24}$ multimers, which assemble around the substrate or into which the substrate diffuses through the pores. These huge multimeric complexes promote either (iii-a) folding of unfolded or partially folded substrate or (iii-b) proteolysis of misfolded substrate protein. (iv) Interaction of $DegP_{12}$ or $DegP_{24}$ with the outer membrane promotes insertion of the correctly folded OMP. (v) Loss of substrate through proteolysis or membrane insertion stabilizes $DegP_6$ and triggers disassembly of $DegP_{12}$ and $DegP_{24}$ to complete the cycle. One attractive feature of this model is that it explains how the DegP catalytic chaperone cycle could function in an ATP-independent fashion.

One hallmark of an interesting structural model is that it poses as many new questions as it answers, and the $DegP_{24}$ and $DegP_{12}$ structures are no exception. For example, how do $DegP_{12}$ and $DegP_{24}$ make the decision whether or not to degrade a substrate protein? One explanation is that the mechanism of action of chaperone activity is merely to assemble around fully folded OMP monomers in order to protect them during transit across the periplasmic space. The rapid folding kinetics or tightly folded structure of OMPs could intrinsically protect them from degradation—a sort of "sink-or-swim" mechanism for promoting protein folding. However, DegP has also been reported to have non-OMP substrates (Spiess et al., 1999); is the mechanism of action toward these proteins the same? In addition, what is the timing of interaction with unfolded, newly translocated protein in periplasm? We could imagine that DegP multimers assemble around unfolded, newly translocated substrate proteins as they emerge from the cytoplasm through the translocon pore in order to ensure that these proteins fold correctly in the harsh folding environment of the periplasm. How does DegP distinguish between the inner and outer membranes in promoting insertion of outer membrane proteins? The surfaces of both the inner and outer membranes are negatively charged and could therefore interact with DegP. However, incorrect insertion of β-barrel pores into the cytoplasmic membrane would result in the rapid collapse of the membrane potential and loss of accumulated metabolites from the cytoplasm. One possibility is that DegP interacts directly with the OMP translocation machinery, which consists of the proteins YaeT, NlpB, SmpA, YfgL, and YfiO. Finally, do other proteins that carry out their functions in the ATP-free environment of the periplasm, work by a similar mechanism of action? We look forward to the answers to these and other questions posed by this very remarkable structure.

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